Taking the Gonococcus-Human Relationship to a Whole New Level: Implications for the Coevolution of Microbes and Humans

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ABSTRACT While horizontal gene transfer occurs frequently among bacterial species, evidence for the transfer of DNA from host to microbe is exceptionally rare. However, the recent report by Anderson and Seifert [mBio 2(1):e00005-11, 2011] provides evidence for such an event with the finding that 11% of *Neisseria gonorrhoeae* strains harbor a 685-bp sequence that is 98 to 100% identical to the human long interspersed nuclear element L1. While the function of this element in gonococci remains unclear, this finding significantly impacts our consideration of the coevolution of hosts and microbes, particularly that of humans and pathogens.

From the time of our birth, we are in constant contact with microbes. Most of these interactions are benign and can even promote good health. On occasion, however, certain pathogenic microbes or their by-products can cause disease that seriously impacts our well-being or even cause our death. Microbe-human interactions have occurred over the history of the evolution of *Homo sapiens* and, most likely, that of our evolutionary precursors. A question in evolution has been whether genetic exchange between microbes and their hosts is possible and whether such exchanges impact the biological processes of the recipient. While there has been considerable evidence for horizontal gene transfer (HGT) among bacterial species and examples of insertion of microbial DNA (especially that of animal viruses) within human chromosomal DNA sequences, heretofore there has been less evidence for the presence of human DNA sequences in bacterial DNA. In the exciting report by Anderson and Seifert published in *mBio* (1), this evidence is now at hand. Briefly, their finding of a 685-bp sequence within the chromosome of 11% of *Neisseria gonorrhoeae* strains now makes us think of the coevolution of humans and microbes at a whole new and most interesting level.

N. gonorrhoeae is a strict human pathogen that has caused the sexually transmitted infection termed gonorrhea for thousands of years. Early accounts of gonococcus-human interactions can be surmised by a passage in the Book of Leviticus (chapter 15, verses 1 to 3) in the Old Testament that essentially warns women to avoid men with discharges, as they are unclean. Since the theme of this passage is on sexual practices, this discharge most likely refers to the purulent exudate from the penis that is typically seen as a result of the inflammatory response in males with gonorrhea. Later writings, particularly by the second century Greek physician Galen, who coined the term gonorrhea ("flow of seed"), support the notion that this is an ancient disease. Hence, *N. gonorrhoeae* and humans have had an intimate relationship for thousands of years. Not surprisingly, this relationship has resulted in the ability of gonococci to resist many natural host defenses that normally function at sites in the human body that it infects.

With the report of Anderson and Seifert (1), we now see that this relationship is at a higher level than we previously thought. By bioinformatic analysis of genome sequences for 14 unrelated (nonclonal) gonococcal strains, Anderson and Seifert discovered a 685-bp sequence that was 98 to 100% identical to the long interspersed nuclear element L1; importantly, they ruled out the possibility of contamination. In all cases, this inserted human DNA sequence in the gonococcal chromosome was adjacent to the *irg4* gene, which encodes a phage transposase. Interestingly, the sequence was expressed with the production of mRNA detectable by reverse transcription-PCR (RT-PCR), so the possibility that strains bearing the sequence can make a human protein exists; this is discussed below. An expanded search revealed that 11% of gonococcal strains harbor the human L1 sequence, which is termed nL1 in the gonococcus. The nL1-bearing strains are distinct in many ways, so it is unlikely that they are clonally related. Screening of *Neisseria meningitidis* and common commensal neisserial strains showed that the nL1 sequence was absent in these species, although more strains should be examined to confirm that the HGT event was indeed gonococcus specific. Nevertheless, from an evolutionary perspective, this conclusion is important because gonococci split from meningococci and commensal *Neisseria* spp. (including *N. meningitidis*) only recently, suggesting that the acquisition of the nL1 sequence by gonococci occurred after this divergence. Since it is not universally present in gonococci, we can also deduce that the transfer event occurred rather recently.

Important questions and directions for future research were clearly stated by Anderson and Seifert (1) and demand attention: how did gonococci acquire this sequence? Does it perform any function for the host gonococcus? With respect to the first question, as suggested by the authors, it is most likely that acquisition of the nL1 sequence occurred by transformation. Gonococci are readily transformable at high frequencies by chromosomal DNA, but they usually take up their own DNA, which can be enhanced by a sequence-specific uptake system that seems to be strain specific (2), and that of other *Neisseria* species, albeit at slightly reduced frequencies; plasmid transformation frequencies are considerably lower. Transformation likely occurs during infection, as

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suggested by the presence of mosaic genes that have regions of gene sequences resembling those harbored by commensal neisserial species (3). The spread of the nL1 sequence from the original recipient to other gonococci also likely happened via transformation, allowing its propagation within the gonococcal population. Where the donor DNA originated from in the human host and then encountered the recipient gonococcus is a matter of speculation. Was the DNA released by host cells undergoing apoptosis? Controversy exists in the literature as to whether gonococci or meningococci are pro- or antiapoptotic, so until this is sorted out, it is reasonable to keep this possibility open. Anderson and Seifert also suggested that DNA within chromatin-rich neutrophil extracellular traps (NETs) (4) may be the source of the L1 sequence. NETs are an attractive source of the donor DNA, since gonococci can trigger a significant inflammatory response that is highlighted by the presence of large numbers of neutrophils. Some of these neutrophils have intracellular gonococci, seemingly intact and alive, but other gonococci remain extracellular, and these gonococci could be recipients for transformation. However, the presence of NETs in the genital tracts of males or females infected with gonococci needs to be confirmed.

The function, if any, provided by the nL1 element is unknown and a matter of interest. Since it is present in only a minority of strains, any function it provides must not be essential when one looks at the overall population structure of *N. gonorrhoeae*. Perhaps it tweaks some process in those strains. The gonococcal L1 element (nL1) would encode 164 amino acids of the L1 open reading frame nucleic acid binding protein (5), so perhaps it has novel regulatory properties that are absent in gonococci that lack the sequence. Alternatively, perhaps it performs no function at all, and there has been insufficient time through the course of recent evolution for it to be discarded from the chromosome. The construction of isogenic strains differing in the presence of nL1 and transcriptional profiling studies may help to define what role, if any, is played by this human DNA sequence. (As an aside, it did not escape our attention that nL1 was present in strain FA6140, which was the cause of an outbreak of gonorrhea in Durham, NC, in 1983 that was refractory to penicillin therapy and has been infamously linked to the downfall of penicillin for the treatment of gonorrhea [6]). It is important to note that gonococci can cause many clinical forms of disease in males and females, including invasive infections, and that possession of the nL1 sequence was not associated with a strain causing a particular form of disease. Taken together, the function of nL1 in gonococci does not seem to be related to a particular form of gonorrhea and remains a mystery.

Anderson and Seifert (1) have shown us that our relationship with microbes now includes the possibility of HGT from us to them. While the function of the nL1 element in strains like FA6140 and other gonococci is unknown, the work reported in *mBio* gives us reason to expand our thought processes, as we consider the coevolution of humans and microbes and the consequences of HGT from a host (e.g., humans) to microbes (especially pathogens) for the physiologic and, potentially, virulence systems of the recipient. We should expect that HGT events can also be initiated from nonhuman hosts to their host-restricted pathogens and commensals. In sum, HGT events from host to microbe could significantly impact evolutionary biology on a scale that has heretofore not been analyzed. Anderson and Seifert's report is a reminder that basic science studies on bacteria can make important contributions to advancing our knowledge of biology in general.

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